Soil microbial biomass and activity in two eucalypt plantation soils after fertilisation

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Abstract

Immobilisation of fertiliser nitrogen (N) by soil microbes is potentially a sink of N in fertilised soils. Immobilisation can occur through either an increase in microbial biomass or an increase in the concentration of N in the microbes, or both. The potential importance of an increase in microbial biomass for mediating the supply of N to young fertilised Eucalyptus nitens *plantations was examined in soils derived from either siltstone or basalt in Tasmania. Microbial biomass and activity were measured in the top 5 cm of the A-horizon of the siltstone soil 91 days after fertilising at rates of 0, 300 or 600 kg N/ha, and similarly for the basalt soil 180 days after fertilising. Phosphorus (P) was applied at each site to achieve an NP ratio of either 2:1 or 1:1. Microbial biomass was estimated by the substrate-induced respiration method, and relative biomass and activity by a catalase enzyme assay. Results from both methods were reproducible, but neither method indicated a microbial response to the fertiliser additions. It was concluded that an increase in microbial biomass and the concomitant net immobilisation of N by microbes was not an important mechanism for moderating the long-term supply of N from fertiliser in these soils. Further research is needed to determine the significance of a possible increase in N concentration within microbial cells in such soils. However, in soils such as those examined here which are limited by available carbon, any increase in N concentration is probably not of significance.*

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Introduction

Immobilisation of nitrogen (N) by soil microbes affects N availability to plants after fertilisation (Melin and Nìmmik 1988; Raison *et al.* 1992), but the magnitude of this effect can be limited by low availability of carbon (C) for microbial growth (Okereke and Meints 1985). Immobilisation can occur through either an increase in microbial biomass or concentration of N in the biomass, or both.

Enzyme assays are used as indicators of microbial biomass or microbial activity for monitoring the effects on soils of chemicals, disturbance, or other environmental changes (Tabatabai 1994). A variety of enzymes has been examined with varying degrees of success for evaluating relationships with soil microbial biomass, including dehydrogenase, urease, phosphatase, protease, cellulase, and hydrolases of fluorescein diacetate (e.g. Hankin *et al.* 1982; Schurner and Rosswall 1982). A simple and useful assay is that for catalase, an enzyme present in all aerobic and most facultative microbiota and therefore correlated with total biomass in aerobic systems. It has been widely utilised for this

Table 1. Characteristics of the two sites examined. Chemical measurements are from the 0–10 cm soil depth.

Characteristic	Westfield site	Middlesex site
Longitude	146·29'E	145·45'E
Latitude	42·40'S	41·52'S
Elevation (m a.s.l.)	430	620
Dominant tree species in the native forest	*Eucalyptus regnans*	*Eucalyptus delegatensis*
Previous crop	*Pinus radiata*	none
Date fertilised	16.12.92	13.1.93
N rates (kg / ha)	0, 300, 600	0, 300, 600
N:P ratio of fertiliser	2:1	1:1
Field texture	clay-loam	clay-loam
Parent material	siltstone	basalt
Soil classification (Isbell 1996)	mottled, mesotrophic, brown kurosol	snuffy, mesotrophic, brown ferrosol
Organic C (mg / g)	62	86
C:N	11	14
pH*	5.0	4.8
Extractable P (µg / g)†	44	8

* 1:5 soil:water † Bray No. 2 method

purpose in a variety of habitats including wood (Line 1983) and soil (e.g. Trevors 1984).

In eucalypt plantations, combined applications of N and P (phosphorus) fertilisers are being used to promote tree growth. As part of an investigation to monitor environmental effects of N and P availability to these plantations, we wished to determine whether fertiliser applications affected soil microbial biomass or activity, since any changes might, in turn, affect the release of these nutrients to the plants.

Methods

Sites and fertiliser treatments

Site characteristics and some soil properties of the two sites sampled are shown in Table 1. Both sites receive about 2000 mm rain annually. In 1991–92, the sites were cleared of native forest (Middlesex) or plantation (Westfield), wood debris and most of the litter layer. In March 1992, they were cultivated in rows about 3.6 m apart using a single-tined deep ripper and disc harrows. This operation left a raised mound (1–1.5 m wide and 10–50 cm high) into which, in October 1992, tree seedlings were planted about 2.5 m apart within rows. The area between mounds was not cultivated, but the clearing operations left a mosaic of litter and surface soil that had been scraped at the surface. Two months after planting, ammonium sulphate and triple superphosphate were broadcast in fertilised treatments (Table 1) in a randomised block design with four replicates. Plots were 18–25 m wide and 22–25 m long.

Sample collections

From the uncultivated inter-row areas, twelve 0–5 cm A-horizon soil samples were bulked per plot for analysis of microbial biomass and activity. The 0, 300 and 600 kg N / ha treatments were sampled at Westfield in March 1993 (91 days after fertilising), and at Middlesex in July 1993 (180 days after fertilising). A similar collection, but from only the unfertilised treatment, was taken in April 1993 (133 days after fertilising) to determine respiration responses to additions of glucose, N and P in the laboratory.

Respiration response to glucose, nitrogen and phosphorus additions

All soil collected in April from the unfertilised treatment was bulked, mixed and divided into five portions. The water content of fresh soil was measured by weight loss at 105·C for 17 h. Deionized water (200 ml / kg) was mixed with each portion of soil, together with glucose (C; 4 mg glucose / g fresh soil); C plus $(NH_4)_2SO_4$ (C+N; 0.6 mg N / g fresh soil); K_2HPO_4 (C+P; 0.3 mg P / g fresh soil); or C+N+P (all at previously mentioned concentrations). Controls were prepared containing only water. Measurements were completed within 24 h of sample collection.

Microbial biomass

Microbial biomass was measured by the substrate-induced respiration method of Anderson and Domsch (1978). Fresh soil (300 g fresh) was mixed with 4 mg glucose / g soil and spread on petri dishes to 1.5 cm depth. The petri dishes were enclosed in a 3.0 l air-tight jar and incubated at 21.5·C. Over a period of 2 h, samples of gas were taken and concentrations of CO_2 measured by gas chromatography. Rates of CO_2 production (x, ml CO_2 / h / 100 g dry weight soil) were assumed to be related to microbial biomass carbon (y, mg C / 100 g dry weight soil) by $y = 40.4x + 0.37$.

Microbial activity

A catalase enzyme assay was used as an index of microbial biomass and its activity. Fresh soil (5 g) was continuously and gently mixed with 50 ml 0.05 M phosphate buffer (pH 7.0) which contained 0.02% H_2O_2. A calibrated O_2 electrode was placed in a mechanically stirred solution to measure the rate of O_2 production during a two-minute period at room temperature.

Results and discussion

Additions of glucose increased rates of respiration from 0.47 to about 1.24 ml CO_2 / h / 100 g soil, irrespective of separate additions of either N (with sulphur) or P (with potassium). These results confirmed that, for the siltstone soil, the activity of the microbial biomass was limited by available C (Table 2), which is a prerequisite of the substrate-induced respiration method for measuring microbial biomass (Anderson and Domsch 1978). Combined applications of glucose, N and P resulted in a 123% increase in the rate of respiration above that of glucose alone (Table 2), which suggests that if there were adequate available C in the field, the potential existed for at least a short-term increase in microbial biomass after combined applications of these nutrients.

Table 2. Production of CO_2 by siltstone soil after additions of C, N and P in the laboratory in various combinations (refer to the text for details of the C, N and P additions). Values in the same column followed by the same letter are not significantly different (L.S.D. = 0.03 at P = 0.05, n = 4). Soil was taken from the 0–50 mm depth of the unfertilised treatment.

Laboratory treatment	Rate of CO_2 production (ml CO_2 / h / 100 g soil)
Control	0.47 a
C	1.40 b
CN	1.13 b
CP	1.29 b
CNP	2.76 c

In contrast to the laboratory experiment, neither microbial biomass nor catalase activity was affected by fertilisation in the field for either soil (Table 3). Confidence in the analytical procedures used here to determine microbial C is provided by the ratio of microbial C to total C, which for a soil in the United Kingdom (on which detailed C dynamics had been studied) was in the range 0.01 to 0.03 (Jenkinson 1990). For the soils used in this study, the ratio was in the range 0.016 to 0.023. The values of microbial biomass (Table 3) were also within the ranges of several other studies, including many agricultural and forest soils in Germany (10–1090 mg C / 100 g soil, Anderson and Domsch

Table 3. Microbial biomass and catalase activity in two soils with three rates of N and P fertilisation.

N:P applied (kg / ha)	Microbial biomass (mg C / 100 g soil)	Catalase activity (ppm O_2 / min / 100 g soil)
Siltstone soil (Westfield)		
0:0	121	0.79
300:150	140	0.77
600:300	117	0.91
L.S.D. (P = 0.05)	32	0.45
Basalt soil (Middlesex)		
0:0	143	1.82
300:300	125	1.54
600:600	154	1.57
L.S.D. (P = 0.05)	45	0.78

1978; Hintze *et al.* 1994) and an organic loam soil from a *Eucalyptus pauciflora* forest (50–130 mg C / 100 g soil, Hossain *et al.* 1995). Values of catalase activity cannot be quantitatively compared with those in other studies because the method has not been standardised and calibrations with actual biomass are not available. Nevertheless, neither the substrate-induced respiration method nor the catalase enzyme assay suggests a detrimental effect of fertilisation on microbial biomass or its activity.

Although there is substantial evidence that at least some fertiliser N is immobilised in microbial biomass after fertilisation of forest soils and litter layers (e.g. Melin and Nìmmik 1988; Vitousek and Andariese 1986), very few studies of this topic have specifically measured both microbial biomass and its N content. Some studies draw inferences about immobilisation by measuring related fluxes; for example, rates of respiration (Foster *et al.* 1980) or N mineralisation (Adams and Attiwill 1983; Carlyle 1995; Raison *et al.* 1992), which commonly increase for considerable periods after fertilisation. In the only comparative study of this topic in a eucalypt forest, Hossain *et al.* (1995) found that microbial biomass was increased 47% in the top 0–25 mm soil by N and P fertilisers two and a half years after a combined application of these nutrients, but there was no effect of the combined application in the 25–50 mm depth, and the amount of N contained in the microbial biomass was unaffected in both depths. This result suggests a fertiliser-induced decrease in the concentration of N in microbial biomass in the top 2.5 cm of soil—an unexpected result because the C:N ratio in forest soils and litter decreases after fertilisation (Melin and Nìmmik 1988).

The current study was not sufficiently comprehensive to determine precisely the role of the microbial biomass after fertilisation, particularly during the weeks immediately following fertilisation. However, results suggest that there was no increase in microbial biomass 91 days after fertilisation at one site, or after 180 days at another site, and that the size and activity of the biomass was at least carbon limited. We also speculate that because the biomass was C limited it probably had relatively low C:N and C:P ratios. These low ratios would have minimised the likelihood that substantial amounts of N or P were immobilised, further decreasing their availability to plants. A thorough study of microbial biomass and its N and P content is needed to better understand the role of microbes in mediating nutrient supply to trees after fertilisation. The potential for uptake of organic N also warrants attention because of a recent suggestion that, in some ecosystems, N can be recycled from decomposing litter without being mineralised (Northup *et al.* 1995).

Results from the present study support a working hypothesis that immobilisation of N and P by soil microbes in these soils is unimportant for mediating the supply of N and P to trees because the effect is either ephemeral or insignificant on time scales relevant to tree growth.

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